IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING NATIONAL PHASE OF PCT APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495

To: Hon. Commissioner of Patents
Washington, D.C. 20231

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

Atty Dkt: PM 0276612 /068800
M# /Client Ref.

From: Pillsbury Winthrop LLP, IP Group:

Date: January 16, 2001

This is a **REQUEST** for **FILING** a PCT/USA National Phase Application based on:

1. International Application	2. International Filing Date	3. Earliest Priority Date Claimed
PCT/GB99/02247	13 July 1999 (13.07.99)	13 July 1998 (13.07.98)
⇧ country code	Day MONTH Year	Day MONTH Year (use item 2 if no earlier priority)

4. Measured from the earliest priority date in item 3, this PCT/USA National Phase Application Request is being filed within:

(a) ☐ 20 months from above item 3 date (b) ☒ 30 months from above item 3 date,

(c) Therefore, the due date (unextendable) is January 13, 2001

5. Title of Invention SILICON-CONTAINING LINKERS FOR NUCLEIC ACID MASS MARKERS

6. Inventor(s) Gunter Schmidt, Andrew Hugin Thompson, and Robert Alexander Walker Johnstone

Applicant herewith submits the following under 35 U.S.C. 371 to effect filing:

7. ☒ Please immediately start national examination procedures (35 U.S.C. 371 (f)).

8. ☒ **A copy of the International Application** as filed (35 U.S.C. 371(c)(2)) is transmitted herewith (file if in English but, if in foreign language, file only if not transmitted to PTO by the International Bureau) including:

a. ☒ Request;
b. ☒ Abstract;
c. 16 pgs. Spec. and Claims;
d. 6 sheet(s) Drawing which are ☐ informal ☒ formal of size ☒ A4 ☐ 11"

9. ☒ **A copy of the International Application has been transmitted by the International Bureau.**

10. **A translation of the International Application** into English (35 U.S.C. 371(c)(2))
a. ☐ is transmitted herewith including: (1) ☐ Request; (2) ☐ Abstract;
(3) _____ pgs. Spec. and Claims;
(4) _____ sheet(s) Drawing which are:
☐ informal ☐ formal of size ☐ A4 ☐ 11"
b. ☒ is not required, as the application was filed in English.
c. ☐ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.
d. ☐ Translation verification attached (not required now).

11. ☒ **PLEASE AMEND** the specification before its first line by inserting as a separate paragraph:
a. ☒ --This application is the national phase of international application PCT/GB99/02247 filed July 13, 1999 which designated the U.S, and that international application ☒ was ☐ was not published under PCT Article 21(2) in English.--
b. ☐ --This application also claims the benefit of U.S. Provisional Application No. 60/ _____, filed _____ .--

RE: USA National Filing of PCT /GB99/02247

500 Rec'd PCT/PTO 16 JAN 2001

12. ☒ Amendments to the claims of the International Application **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., before 18th month from first priority date above in item 3, are transmitted herewith (file only if in English) including:**

13. ☒ PCT Article 19 claim amendments (if any) have been transmitted by the International Bureau

14. ☐ Translation of the amendments to the claims **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., of claim amendments** made before 18th month, **is attached (required by 20th month from the date in item 3 if box 4(a) above is X'd, or 30th month if box 4(b) is X'd, or else amendments will be considered canceled).**

15. **A declaration of the inventor** (35 U.S.C. 371(c)(4))
a. ☐ is submitted herewith ☐ Original ☐ Facsimile/Copy
b. ☒ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.

16. **An International Search Report (ISR):**
a. Was prepared by ☒ European Patent Office ☐ Japanese Patent Office ☐ Other
b. ☒ has been transmitted by the international Bureau to PTO.
c. ☒ copy herewith (4 pg(s).) ☐ plus Annex of family members (___ pg(s).).

17. **International Preliminary Examination Report** (IPER):
a. ☒ has been transmitted (if this letter is filed after 28 months from date in item 3) in English by the International Bureau with Annexes (if any) in original language.
b. ☒ copy herewith in English.
c.1 ☒ IPER Annex(es) in original language ("Annexes" are amendments made to claims/spec/drawings during Examination) including attached amended:
c.2 ☒ Specification/claim pages #4 claims #1-30
Dwg Sheets #
d. ☐ Translation of Annex(es) to IPER **(required by 30th month due date, or else annexed amendments will be considered canceled).**

18. **Information Disclosure Statement** including:
a. ☒ Attached Form PTO-1449 listing documents
b. ☒ Attached copies of documents listed on Form PTO-1449
c. ☒ A concise explanation of relevance of ISR references is given in the ISR.

19. ☐ **Assignment** document and Cover Sheet for recording are attached. Please mail the recorded assignment document back to the person whose signature, name and address appear at the end of this letter.

20. ☐ Copy of Power to IA agent.

21. ☐ **Drawings** (complete only if 8d or 10a(4) not completed): ___ sheet(s) per set: ☐ 1 set informal; ☐ Formal of size ☐ A4 ☐ 11"

22. Small Entity Status ⌀ ☐ is **Not** claimed ☒ is claimed (**pre**-filing confirmation required)
22(a) (n/a) (No.) Small Entity Statement(s) enclosed (since 9/8/00 Small Entity Statements(s) not essential to make claim)

23. **Priority** is hereby claimed under 35 U.S.C. 119/365 based on the priority claim and the certified copy, both filed in the International Application during the international stage based on the filing in (country) Great Britain of:

	Application No.	Filing Date		Application No.	Filing Date
(1)	9815164.0	13 July 1998	(2)		
(3)			(4)		
(5)			(6)		

a. ☒ See Form PCT/IB/304 sent to US/DO with copy of priority documents. If copy has not been received, please proceed promptly to obtain same from the IB.
b. ☐ Copy of Form PCT/IB/304 attached.

24. Attached:

RE: USA National Filing of PCT/GB99/02247

25. **Preliminary Amendment:**

25.5 Per Item 17.c2, **cancel original** pages #_____, claims #_____, Drawing Sheets #

26. **Calculation of the U.S. National Fee (35 U.S.C. 371 (c)(1)) and other fees is as follows:**

Based on amended claim(s) per above item(s) ☐ 12, ☐ 14, ☐ 17, ☐ 25, ☐ 25.5 (hilite)

Total Effective Claims	30	minus 20 =	10	x $18/$9	=	$90	966/967
Independent Claims	3	minus 3 =	0	x $80/$40	=	$0	964/965
If any proper (ignore improper) Multiple Dependent claim is present,				add$270/$135		+135	968/969

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)): →→ **BASIC FEE REQUIRED, NOW** →→→→

A. If country code letters in item 1 are **not** "US","BR","BB","TT","MX","IL" "NZ", "IN" or "ZA"

See item 16 re:

1.	Search Report was not prepared by EPO or JPO - - - - - - - - -	add$1000/$500	960/961
2.	Search Report was prepared by EPO or JPO - - - - - - - - - - - -	add$860/$430 +430	970/971

SKIP B, C, D AND E UNLESS country code letters in item 1 are "US","BR","BB","TT","MX","IL", "NZ", "IN" or "ZA"

(X) →	☒ B.	If USPTO did not issue both International Search Report (ISR) and (if box 4(b) above is X'd) the International Examination Report (IPER), -	add$970/$485	+_____ 960/961
(only) (one) → (of) (these) (4) → (boxes)	☐ C.	If USPTO issued ISR but not IPER (or box 4(a) above is X'd), -	add$710/$355	+0 958/959
	☐ D.	If USPTO issued IPER but IPER Sec. V boxes not all 3 YES, -	add$690/$345	+0 956/957
→	☐ E.	If international preliminary examination fee was paid to USPTO and Rules 492(a)(4) and 496(b) satisfied (IPER Sec. V all 3 boxes YES for all claims), - - - - - - - - - - - - -	add $100/$50	962/963 +0
27.			**SUBTOTAL =**	$ 655.00

28. If Assignment box 19 above is X'd, add Assignment Recording fee of ----$40 +0 (581)

29. Attached is a check to cover the - **TOTAL FEES** $ 655.00

Our Deposit Account No. 03-3975
Our Order No. _____ C# | _____ M#

CHARGE STATEMENT: The Commissioner is hereby authorized to charge any fee specifically authorized hereafter, or any missing or insufficient fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under Rules 16-18 and 492 (missing or insufficient fee only) now or hereafter relative to this application and the resulting Official document under Rule 20, or credit any overpayment, to our Account/Order Nos. shown above for which purpose a duplicate copy of this sheet is attached.

This CHARGE STATEMENT does not authorize charge of the issue fee until/unless an issue fee transmittal form is filed

Pillsbury Winthrop LLP
Intellectual Property Group

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By Atty: Robin L. Teskin **Reg. No.** 35,030
Sig: _____ **Fax:** (202) 822-0944
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NOTE: File in duplicate with 2 postcard receipts (PAT-103) & attachments.

SILICON-CONTAINING LINKERS FOR NUCLEIC ACID MASS MARKERS

This invention concerns compounds which comprise mass markers for detection by mass spectrometry. The invention relates to methods for characterising nucleic acids or other molecules by labelling with markers that are cleavably detachable from their associated nucleic acid and that are detectable by mass spectrometry. Specifically this invention relates to improved methods of detaching mass labels from their associated nucleic acids or other molecules of interest.

PCT/GB98/00127 describes arrays of cleavable labels that are detectable by mass spectrometry which identify the sequence of a covalently linked nucleic acid probe. These mass labels have a number of advantages over other methods of analysing nucleic acids. At present commercially favoured systems are based on fluorescent labelling of DNA. Fluorescent labelling schemes permit the labelling of a relatively small number of molecules simultaneously, typically 4 labels can be used simultaneously and possibly up to eight. However the costs of the detection apparatus and the difficulties of analysing the resultant signals limit the number of labels that can be used simultaneously in a fluorescence detection scheme. An advantage of using mass labels is the possibility of generating large numbers of labels which have discrete peaks in a mass spectrum allowing similar numbers of distinct molecular species to be labelled simultaneously. Fluorescent dyes are expensive to synthesise whereas mass labels can comprise relatively simple polymers permitting combinatorial synthesis of large numbers of labels at low cost.

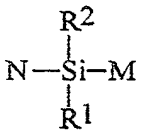
A feature of the mass labelling techniques disclosed in PCT/GB98/00127 is the need for linker groups that covalently link a mass marker to its corresponding nucleic acid. These linkers must permit the mass marker to be separated from its nucleic acid prior to detection within a mass spectrometer. It is desirable that the cleavage of the label from its nucleic acid be performed in-line with a mass spectrometer, possibly after some in-line pre-fractionation step such as capillary electrophoresis. It is also desirable that this in-line cleavage step does not require a complex interface with the mass spectrometer to enable this step to occur. Ideally

linkers should cleave at some predetermined point within existing instruments without any modification to the instrument beyond changes of normal operating parameters.

Linkers should cleave without damaging associated nucleic acids hence reducing noise in the mass spectrum from nucleic acid fragmentation. Linkers should all cleave under the same conditions to ensure all labels can be analysed simultaneously and quantitatively.

It is an object of this invention to provide linkers that have the desired features disclosed above which are compatible with existing mass spectrometers particularly electrospray ionisation and tandem mass spectrometry.

Accordingly, the present invention provides a compound having the following formula (I):



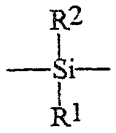
(I)

wherein M comprises a mass marker, N comprises a nucleic acid and R^1 and R^2 are substituents selected such that when the compound reacts with an electron donating moiety, either N or M cleaves from the Si atom in preference to R^1 and R^2.

Compounds with the formula (I) shown above meet the specification discussed above. The molecule is stable during synthesis and can be cleaved under mild conditions in an electrospray ion source or in the collision chamber of a tandem mass spectrometer in the presence of an appropriately reactive gaseous electron donating moiety, such as ammonia. The reactive gas participates in a novel gas phase reaction with the linker resulting in the cleavage of the linker.

The present invention also provides a method for characterising an analyte, which method comprises:

(a) providing a compound in which the analyte is attached by a cleavable linker to a reporter group relatable to the analyte, the linker having the following formula:

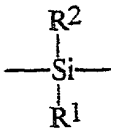


wherein R^1 and R^2 are substituents as defined below;

(b) cleaving the reporter group from the analyte; and

(c) identifying the reporter group, thereby characterising the analyte.

The invention additionally provides use of a linker group in the characterisation of an analyte, to attach a reporter group to the analyte, wherein the linker group is cleavable and has the following formula:



wherein R^1 and R^2 are substituents as defined below.

The invention will now be described in further detail by way of example only, with reference to the accompanying drawings, in which:

Figure 1 depicts the mechanism of cleavage of a linker used in the present invention, by means of a primary amine. Cleavage takes place via a five-co-ordinate intermediate to produce two possible products. The mass spectrum of the charged products is measured;

Figure 2 shows the negative ion mass spectrum of FT23 at 0.35 pmol/µl. This spectrum shows a very clean molecular ion less 1 proton at m/z 729.4;

Figure 3 shows the positive ion mass spectrum of FT23 at 0.35 pmol/µl. This spectrum shows a strong protonated molecular ion peak at m/z 731.5 and a second stronger peak at m/z 753.3 corresponding to a sodium adduct of FT23;

Figure 4 shows the negative ion mass spectrum of FT23 at 0.35 pmol/µl in the presence of a PCR product. The molecular ion is not detectable over the background peaks;

Figure 5 shows the positive ion mass spectrum of FT23 at 0.35 pmol/µl in the presence of a PCR product. This spectrum does not show any of the protonated molecular ion peak at m/z 731.4 or the sodium adduct peak at m/z 753.3. Two additional peaks of significance appear in this spectrum. One peak at m/z 739.2 and a second peak at m/z 755.2. These peaks are believed to be end-products of a novel gas phase cleavage reaction discussed below. The two peaks corresponding to gas phase cleavage products are the only major ion peaks in this spectrum; and

Figure 6 shows a reaction mechanism for ammonia reacting with a TBDMS protective group used in the present invention.

In the methods of the present invention, the analyte is not particularly limited and can be any analyte or molecule of interest, such as a nucleic acid or other molecule. Typically the analyte comprises a biological molecule. In preferred embodiments of the present invention, the biological molecule is selected from a protein, a polypeptide, an amino acid, a nucleic acid (e.g. an RNA, a DNA, a plasmid, a nucleotide or an oligonucleotide), a nucleic acid base, a pharmaceutical agent or drug, a carbohydrate, a lipid, a natural product and a synthetic compound from an encoded chemical library. When the analyte comprises a nucleotide, oligonucleotide or nucleic acid, the nucleotide, oligonucleotide or nucleic acid may be natural,

or may be modified by modifying a base, sugar and/or backbone of the nucleotide, oligonucleotide or nucleic acid. In the compounds of the present invention, the analyte is a nucleic acid, and may be any type of nucleic acid. Preferably, the nucleic acid is of a type as defined above.

The substituents R^1 and R^2 are not especially limited. It is preferred that R^1 and R^2 are selected such that their bond energies to Si are greater than the bond energy of N and/or M to Si to ensure that when the compound is reacted with an electron donating moiety either N or M cleaves from the Si atom in preference to R^1 and R^2, and/or R^1 and R^2 are selected such that their steric bulk is sufficient to ensure that when the compound is reacted with an electron donating moiety either N or M cleaves from the Si atom in preference to R^1 and R^2. Typically, R^1 and R^2 are each independently a hydrogen atom, a halogen atom, a substituted or unsubstituted alkyl group, or a substituted or unsubstituted aryl group. It is particularly preferred that R^1 and R^2 are each independently fluorine, chlorine, bromine, iodine, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl or phenyl groups.

Thus, various substituents may be introduced at the positions R^1 and R^2 including fluorine, chlorine and other halogens, methyl, ethyl and other alkyl groups. Phenyl groups may also be appropriate. Preferably substituents at R^1 and R^2 should be stable during synthesis of the marker, during incorporation of the mass label into an oligonucleotide in an automated synthesiser and under mass spectrometry. A wide variety of groups have these properties and may be incorporated into the linker at these positions. It may also be desirable in some embodiments to choose substituents which change the solubility of the linker and alter the rigidity of the linker.

Preferably a covalent linkage is formed in attaching the analyte and/or the reporter group to the cleavable linker. The covalent linkage is not particularly limited provided that the analyte and/or reporter group can readily be attached to the cleavable linker using reactive functionalities attached to the linker and the analyte.

Table 1 below lists some reactive functionalities that may be reacted together to generate a covalent linkage between two entities. Any of the functionalities listed below could be used to form the compounds used in the present invention to permit the linker to be attached to an analyte (such as a nucleic acid or protein) for detection (e.g. by mass spectrometry). If desired, a reactive functionality can be used to introduce a further linking group with a further reactive functionality.

Table 1

Functionality 1	Functionality 2	Resultant Covalent Linkage
$-NH_2$	-COOH	-CO-NH-
$-NH_2$	-NCO	-NH-CO-NH-
$-NH_2$	-NCS	-NH-CS-NH-
$-NH_2$	-CHO	$-CH_2$-NH-
$-NH_2$	$-SO_2Cl$	$-SO_2$-NH-
$-NH_2$	-CH=CH-	-NH-CH_2-CH_2-
-OH	$-OP(NCH(CH_3)_2)_2$	-OP(=O)(O)O-

It should be noted that some of the reactive functionalities above or their resultant covalent linkages might have to be protected prior to introduction into an oligonucleotide synthesiser. Preferably unprotected ether, ester, thioether and thioesters, amine and amide bonds are to be avoided as these are not stable in an oligonucleotide synthesiser. A wide variety of protective groups are known in the art to protect linkages from unwanted side reactions.

A short alkyl linkage is appropriate to link the mass marker to the linker, although a wide variety of linkages are available which can be used to link a mass marker to a linker.

The reporter group used in the present invention is not especially limited and may be any group, provided that it is readily detectable and can se related to an analyte to identify the analyte. Typically, the reporter group is a mass marker, that is detectable by mass spectrometry. Other appropriate reporters include fluorophores, radiolabels, chemiluminescent

labels, and electron capture labels. In the compounds of the present invention, the reporter group comprises a mass marker.

In preferred embodiments of the present invention, mass markers disclosed in PCT/GB98/00127, PCT/GB98/03842, GB 9815166.5 and GB 9826159.7 can be employed. The content of these applications is incorporated by reference. PCT/GB98/00127 and PCT/GB98/03842 disclose poly-ether mass markers which are thermally stable, chemically inert and fragmentation resistant compounds, and which can be substituted with a variety of groups to alter properties such as solubility and charge. These mass markers are also preferred for use in the present invention and the content of this application is incorporated by reference. GB 9826159.7 discloses markers which comprise two components, which may be poly-ethers, which are analysed by selected reaction monitoring. These are particularly preferred mass markers for use in the present invention. GB 9815166.5 discloses mass markers that bind metal ions, which are also preferred markers for use with this invention. The content of this application is incorporated by reference. Reporter groups that can be detected by more than one detection means may also be desirable as with, for example, a fluorescent marker that incorporates a radioisotope in its linker and that is detectable by mass spectrometry and reporters of this kind are refereed to as 'multi-mode reporter' groups. Preferred multi-mode reporter groups are detectable by mass spectrometry.

When the mass marker comprises an oligoether or a polyether, the oligoether or polyether may be a substituted or unsubstituted oligo- or poly-arylether. The oligoether or polyether preferably comprises one or more fluorine atom or methyl group substituents, or one or more ^{2}H or ^{13}C isotopic substituents.

It is further preferred that the mass marker comprises a metal ion-binding moiety. Typically, the metal ion-binding moiety comprises a porphyrin, a crown ether, hexahistidine, or a multidentate ligand. Preferably, the metal ion-binding moiety is a bidentate ligand or is EDTA. The metal ion-binding moiety may be bound to a monovalent, divalent or trivalent metal ion. The metal ion is not especially limited. Preferred metal ions include a transition

metal ion, or a metal ion of group IA, IIA or IIIA of the periodic table. Particularly preferred metal ions are Ni^{2+}, Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, or Al^{3+}. The presence of a metal ion on the mass marker increases the sensitivity of detection.

It should be noted that this invention is not limited to the mass markers disclosed in the above applications. Any molecule with the correct features can be used as a mass marker. Desirable features include:

- Easily detachable from DNA
- Fragmentation resistant in mass spectrometer
- Single ion peaks
- Very sensitive detection
- Easily distinguishable from background contamination
- Distinguish from DNA
- Be certain that a mass peak is from a mass label
- Compatible with oligonucleotide synthesiser
- Easy to synthesise in a combinatorial manner to minimise number of chemical steps and the number of reagents necessary to generate large number of labels
- Compatible with existing mass spectrometry instrumentation without requiring physical modification.

Mass labels and their linkers can be attached to a nucleic acid molecule at a number of locations in the nucleic acid. For conventional solid phase synthesisers the 5' hydroxyl of the sugar is the most readily accessible. Other favoured positions for modifications are on the base at the 5' position in the pyrimidines and the 7' and 8' positions in the purines. These would all be appropriate positions to attach a cleavable mass with the linker of this invention.

The 2' position on the sugar is accessible for mass modifications but is more appropriate for small mass modifications that are not to be removed.

The phosphate linkage in natural nucleic acids can be modified to a considerable degree as well, including derivitisation with mass labels.

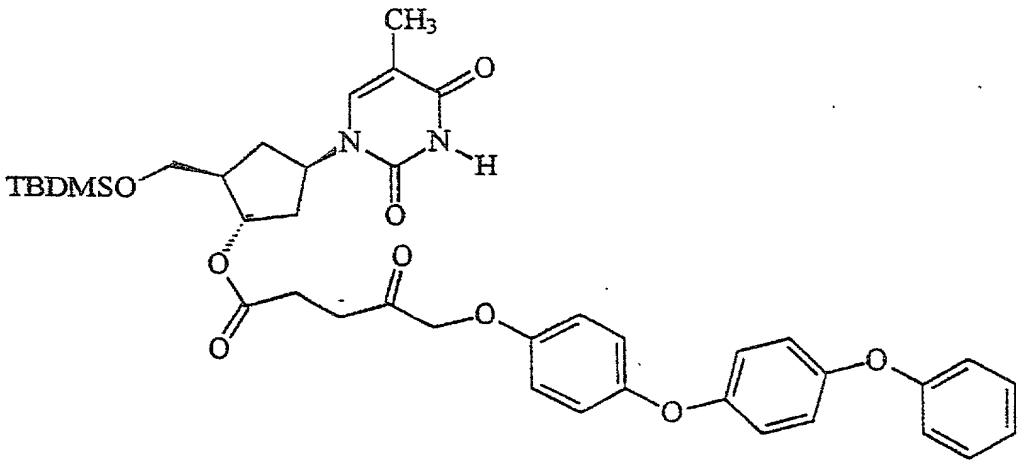
The cleavable linker used in this invention may be cleaved in the ion source of a mass spectrometer by ammonia. However, this invention is not limited to the use of ammonia. Most amines are be capable of separating the mass marker from its cognate oligonucleotide and other nucleophiles may also be used.

The invention will now be described in further detail by way of example only, with reference to the following specific embodiments.

Examples

Synthesis of a mass labelled nucleotide FT23

A thymidine residue with a poly-aryl ether mass label was synthesised as a model compound, shown below:



TBDMS=Tertiary butyl dimethylsilyl

A solution of 5'-O-(tert-butyl dimethylsilyl)-3'-succinyl-thymidine (288 mg, 0.5 mmol) in dichloromethane (3 ml) was treated with three drops of pyridine and then dropwise with a

2 M solution of oxalyl chloride (0.3 ml, 0.6 mmol) in dichloromethane. The reaction mixture was stirred for 90 min at room temp. The solution of the formed acid chloride was added dropwise to an ice-cold solution of (4'-phenoxy)-4-phenoxybenzyl alcohol (146 mg, 0.5 mmol) and pyridine (0.3 ml) in dichloromethane (3 ml). Stirring was continued for 4 h at room temperature. The reaction mixture was diluted with ethyl acetate and washed with a 5 % aqueous solution of $NaHCO_3$ and twice with water. The organic phase was dried with sodium sulphate and the solvent was removed under reduced pressure. The residue was purified by flash chromatography with ethyl acetate/n-hexane (1:1) to yield 73 mg (20 %) of FT 23.

^1H NMR ($CDCl_3$): 0.13 (6 H, s); 0.92 (9 H, s); 1.92 (3 H, s); 2.11 (1 H, m); 2.39 (1 H, m); 2.68 (4 H, s); 3.90 (2 H, d); 4.06 (1 H; d); 5.11 (2 H, s); 5.27 (1 H, d); 6.34 (1 H; m); 6.95-7.37 (13 H, m); 7.35 (1 H, d); 8.27 (1 H, br s).

The product of the synthesis was analysed by ESI MS to determine whether the predicted molecular ions were present. The protonated molecular ion was detected at m/z 731 and the molecular less 1 proton was detected at m/z 729. (Mass spectra shown in Figures 4 and 5 respectively)

Mass Spectrometry Analysis of FT23

All data was acquired on a Platform-LC quadrupole instrument (Micromass Ltd, UK) with an electrospray ionisation source.

Figure 2 shows the negative ion mass spectrum of FT23 at 0.35 pmol/µl. This spectrum shows a very clean molecular ion less 1 proton at m/z 729.4.

Figure 3 shows the positive ion mass spectrum of FT23 at 0.35 pmol/µl. This spectrum shows a strong protonated molecular ion peak at m/z 731.5 and a second stronger peak at m/z 753.3 corresponding to a sodium adduct of FT23. Without being bound by theory, it is believed that the poly-ether mass label with the succinate linker is behaving in a similar manner to a crown ether and is binding strongly to sodium to generate these sodium adducts.

Figure 4 shows the negative ion mass spectrum of FT23 at 0.35 pmol/µl in the presence of a PCR product. The molecular ion is not detectable over the background peaks.

Figure 5 shows the positive ion mass spectrum of FT23 at 0.35 pmol/µl in the presence of a PCR product. This spectrum does not show any of the protonated molecular ion peak at m/z 731.4 or the sodium adduct peak at m/z 753.3. Two additional peaks of significance appear in this spectrum. One peak at m/z 739.2 and a second peak at m/z 755.2. These peaks are believed to be end-products of a novel gas phase cleavage reaction discussed below. The two peaks corresponding to gas phase cleavage products are the only major ion peaks in this spectrum.

FT23 is a thymidine derivative which has been protected on the 5' hydroxyl using a tert-butyl dimethylsilyl (TBDMS) protective group. Without being bound by theory, it is believed that in this invention a small quantity of ammonia present in the sample, introduced with the PCR product reacts with the TBDMS protective group according to the mechanism shown in Figure 6. Water appears to react with the TBDMS group to some extent as well. The chemical reactions which take place do not have any charged intermediates. The reaction is visible in the mass spectrometer because of the sodium ion binding behaviour of the mass label used in these studies. The lack of any protonated ions suggest that sodium binding is very strong and that an excess of sodium ions is present which binds to all of the FT23 present. This gives rise to sodium adducts which have a single positive charge. Nucleophilic attack by ammonia generates a trigonal pyramidal intermediate which is energetically unstable. This intermediate rearranges itself with the loss of formyl amine to generate an ion with m/z 739. Similarly nucleophilic attack by water generates a trigonal pyramidal intermediate which is energetically unstable. This intermediate rearranges itself with the loss of methane to generate an ion with m/z 755.

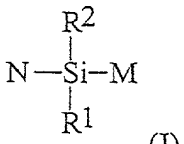
These cleavage product peaks are seen as the majority ions when 0.35 pmol/µl of FT23 is analysed in the positive ion mode in the presence of a PCR product (Figure 5) but are not

observed when the pure compound is analysed (Figure 3). At higher concentrations of FT23 the singly protonated molecular ion peaks are still detectable in the presence of nucleic acid (not shown). In the negative ion mode the pure compound is readily detected at 0.35 pmol/µl but is not detectable at the same concentration in the presence of a PCR product. Ammonia was present in the buffers of the PCR product. The spectra are interpretable if the low levels of ammonia are cleaving the silyl protective group. The levels of ammonia are limiting - at higher concentrations of FT23 the reaction does not go to completion (not shown) but at the lower concentration of FT23 there is sufficient ammonia to completely cleave the molecular ion.

Figure 1 shows how this chemistry can be adapted for use as a gas phase cleavable linker as discussed above.

CLAIMS:

1. A compound having the following formula (I):



(I)

wherein M comprises a mass marker, N comprises a nucleic acid, and wherein R^1 and R^2 are each independently selected from a hydrogen atom, a halogen atom, a substituted or unsubstituted alkyl group, and a substituted or unsubstituted aryl group such that when the compound reacts with an electron donating moiety, either N or M cleaves from the Si atom in preference to R^1 and R^2.

2. A compound according to claim 1, wherein R^1 and R^2 are each independently selected from fluorine, chlorine, bromine, iodine, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl or phenyl groups.

3. A compound according to claim 1 or claim 2, wherein N comprises a nucleotide or an oligonucleotide.

4. A compound according to claim 3, wherein the nucleotide or oligonucleotide is natural, or is modified by modifying a base, sugar and/or backbone of the nucleotide or oligonucleotide.

5. A compound according to any preceding claim, wherein the mass marker comprises a polyether.

6. A compound according to claim 5, wherein the polyether is a substituted or unsubstituted poly(arylether).

7. A compound according to claim 5 or claim 6, wherein the polyether comprises one or more fluorine atom substituents.

8. A compound according to any preceding claim, wherein the mass marker comprises a metal ion-binding moiety.

9. A compound according to claim 8, wherein the metal ion-binding moiety is a porphyrin, a crown ether, hexahistidine, or a multidentate ligand.

10. A compound according to claim 9, wherein the metal ion-binding moiety is a bidentate ligand or is EDTA.

11. A compound according to any of claims 8-10, wherein the metal ion-binding moiety is bound to a monovalent, divalent or trivalent metal ion.

12. A compound according to claim 11, wherein the metal ion is a transition metal ion, or a metal ion of group IA, IIA or IIIA of the periodic table.

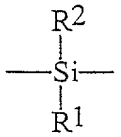
13. A compound according to claim 12, wherein the metal ion is Ni^{2+}, Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, or Al^{3+}.

14. A compound according to any preceding claim, wherein the electron donating moiety is a Lewis base.

15. A compound according to claim 14, wherein the Lewis base is selected from ammonia; a primary, secondary or tertiary amine; a compound containing a hydroxy group; an ether; and water.

16. A method for characterising an analyte, which method comprises:

(a) providing a compound in which the analyte is attached by a cleavable linker to a reporter group relatable to the analyte, the linker having the following formula:



wherein R^1 and R^2 are substituents as defined in any of claims 1, 2, 14 and 15;

(b) cleaving the reporter group from the analyte; and

(c) identifying the reporter group, thereby characterising the analyte.

17. A method according to claim 16, wherein the reporter group is a mass marker identifiable by mass spectrometry.

18. A method according to claim 17, wherein the mass marker is as defined in any of claims 5-13.

19. A method according to any of claims 16-18, wherein the analyte is a nucleic acid.

20. A method according to claim 19, wherein the nucleic acid is as defined in claim 3 or claim 4.

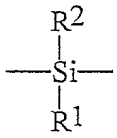
21. A method according to any of claims 16-20, which method further comprises forming a compound as defined in any of claims 1-15, prior to identifying the reporter group.

22. A method according to any of claims 16-21, which method further comprises contacting the linker with an electron donating moiety to cleave off the reporter group.

23. A method according to claim 22, wherein the electron donating moiety is as defined in claim 14 or claim 15.

24. A method according to any of claims 16-23, wherein the reporter group is a mass marker and the method further comprises cleaving off the mass marker in a mass spectrometer.

25. Use of a linker group in the characterisation of an analyte, to attach a reporter group to the analyte, wherein the linker group is cleavable and has the following formula:



wherein R^1 and R^2 are substituents as defined in any of claims 1, 2, 14 and 15.

26. Use according to claim 25, wherein the analyte is a mass marker identifiable by mass spectrometry.

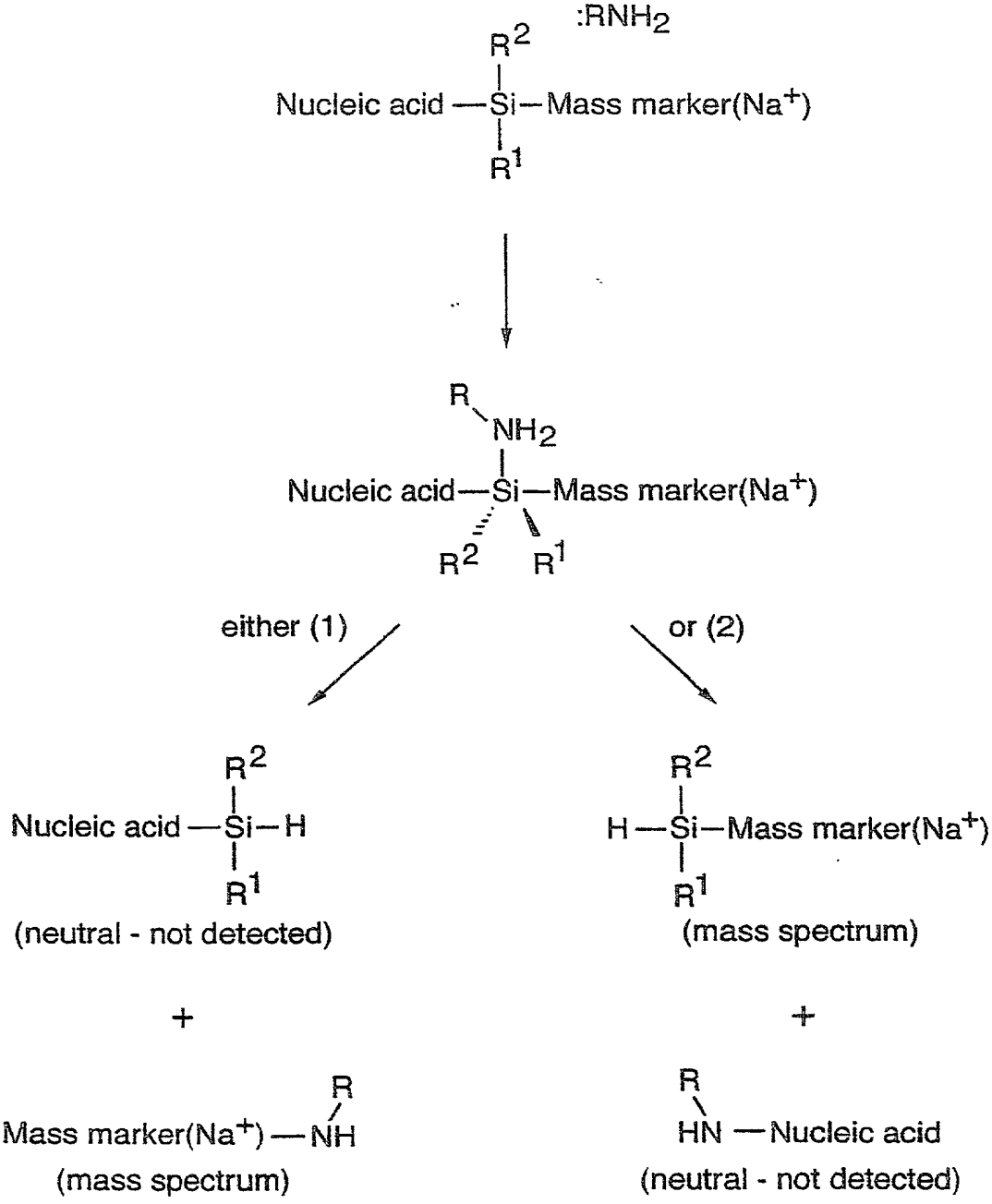
27. Use according to claim 26, wherein the mass marker is as defined in any of claims 5-13.

28. Use according to any of claims 25-27, wherein the analyte is a nucleic acid.

29. Use according to claim 28, wherein the nucleic acid is as defined in claim 3 or claim 4.

30. Use according to any of claims 25-29, wherein the mass marker forms part of a compound as defined in any of claims 1-15.

Fig.1.


:RNH2
Nucleic acid—Si—Mass marker(Na+)
R2
R1
R
NH2
either (1)
or (2)
Nucleic acid—Si—H
(neutral - not detected)
H—Si—Mass marker(Na+)
(mass spectrum)
+
Mass marker(Na+)—NH
(mass spectrum)
HN—Nucleic acid
(neutral - not detected)

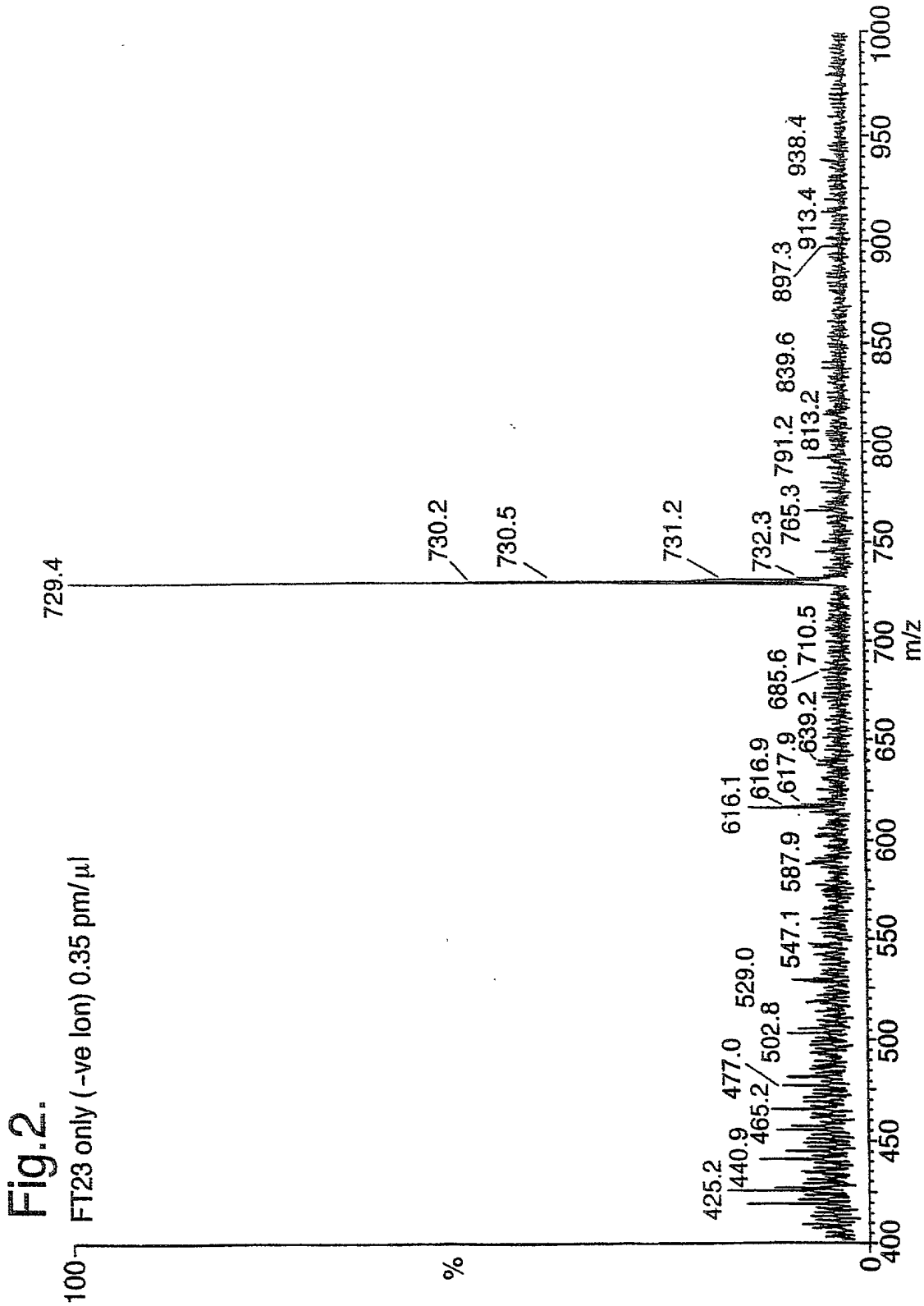

100
%
0
FT23 only (-ve Ion) 0.35 pm/µl
729.4
730.2
730.5
731.2
732.3
765.3
791.2
813.2
839.6
897.3
913.4
938.4
710.5
685.6
639.2
617.9
616.9
616.1
587.9
547.1
529.0
502.8
477.0
465.2
440.9
425.2
400
450
500
550
600
650
700
750
800
850
900
950
1000
m/z

Fig.2.

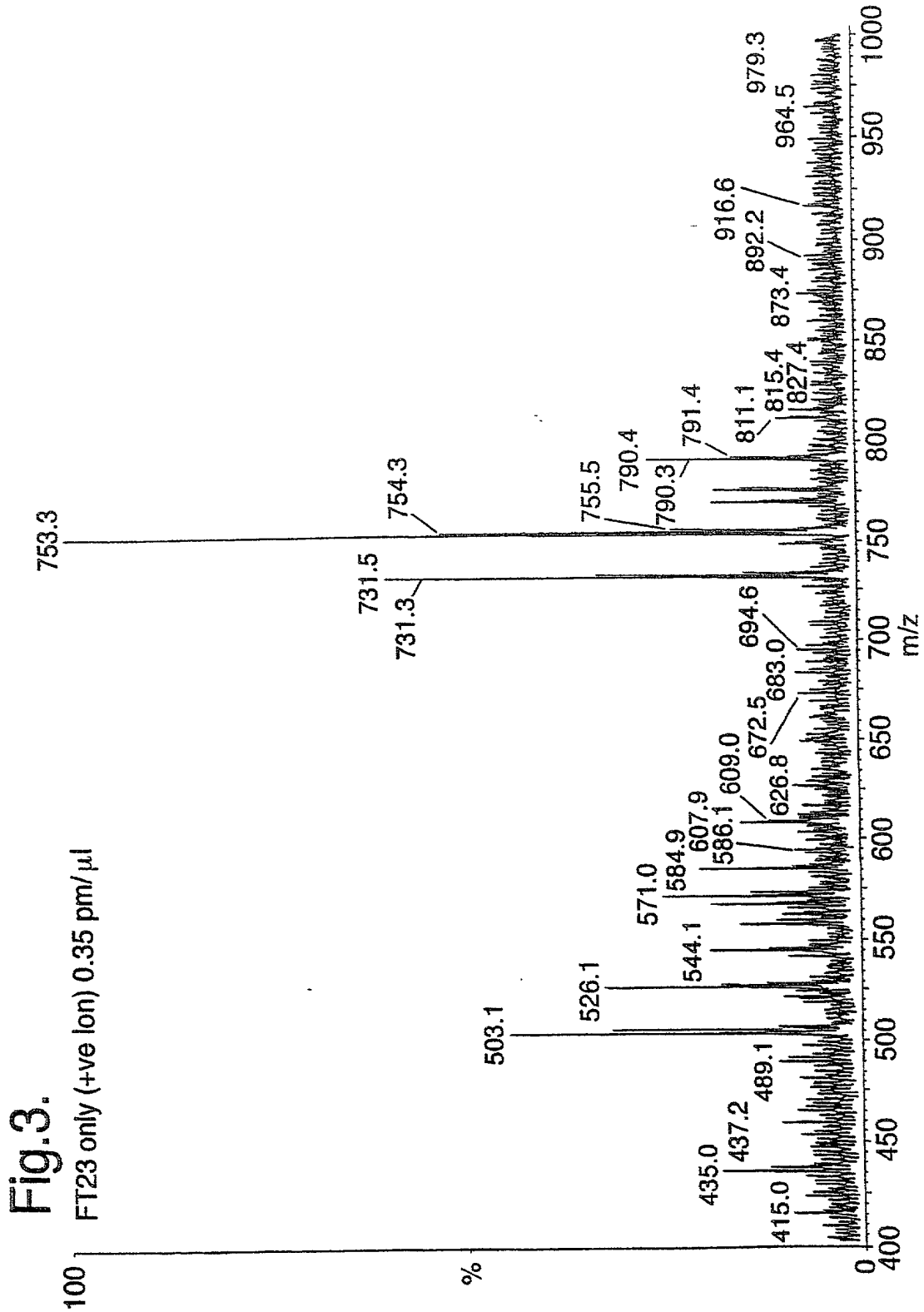
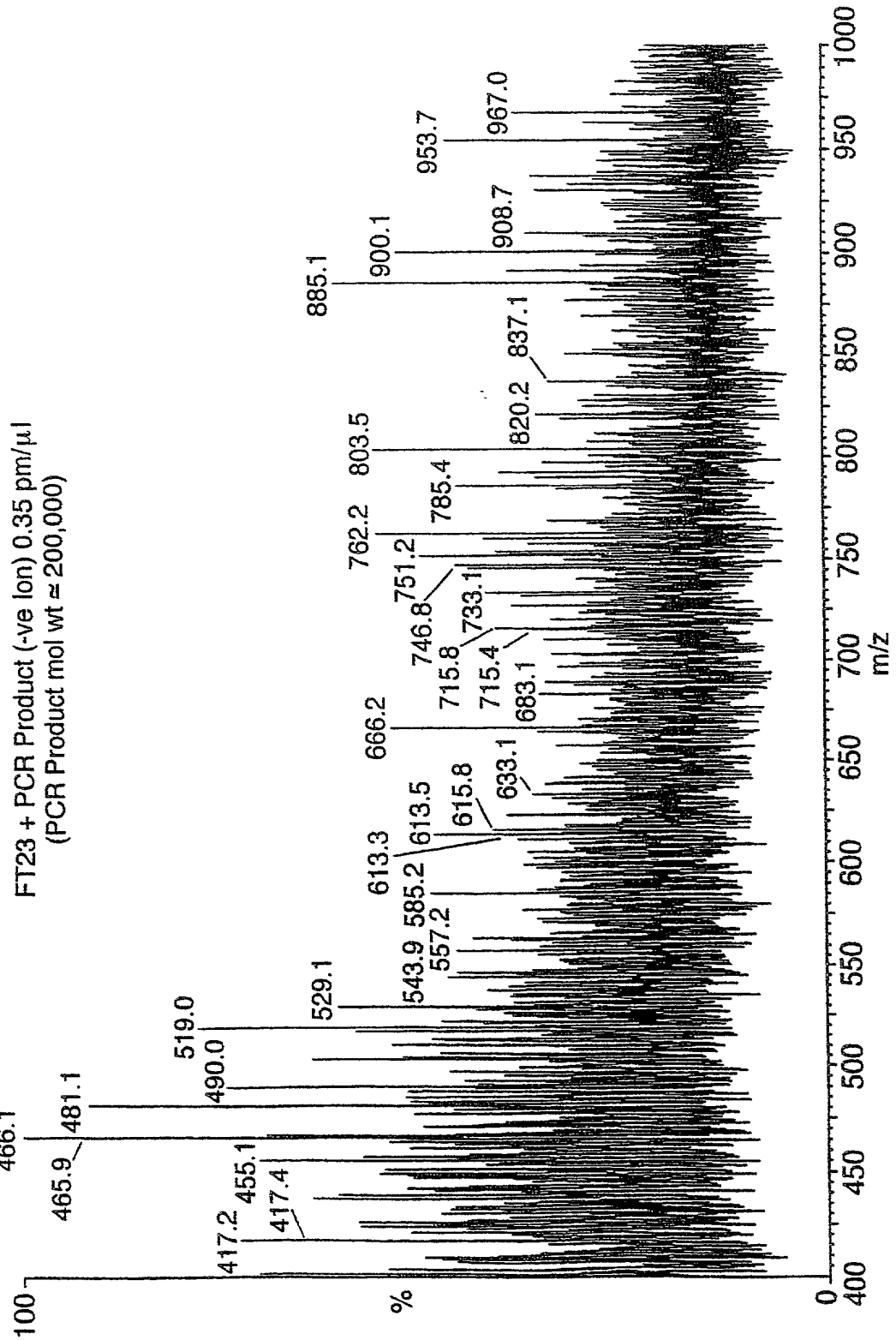

FT23 only (+ve Ion) 0.35 pm/µl
100
%
0
400
450
500
550
600
650
700
750
800
850
900
950
1000
m/z
753.3
754.3
731.5
731.3
755.5
790.4
790.3
791.4
811.1
815.4
827.4
873.4
892.2
916.6
964.5
979.3
694.6
683.0
672.5
626.8
609.0
586.1
607.9
584.9
571.0
544.1
526.1
503.1
489.1
437.2
435.0
415.0

Fig.3.

Fig.4.


FT23 + PCR Product (-ve Ion) 0.35 pm/µl
(PCR Product mol wt ≈ 200,000)
100
%
0
400
450
500
550
600
650
700
750
800
850
900
950
1000
m/z
465.9
466.1
481.1
490.0
519.0
529.1
543.9
557.2
585.2
613.3
613.5
615.8
633.1
666.2
683.1
715.4
715.8
733.1
746.8
751.2
762.2
785.4
803.5
820.2
837.1
885.1
900.1
908.7
953.7
967.0
417.2
455.1
417.4

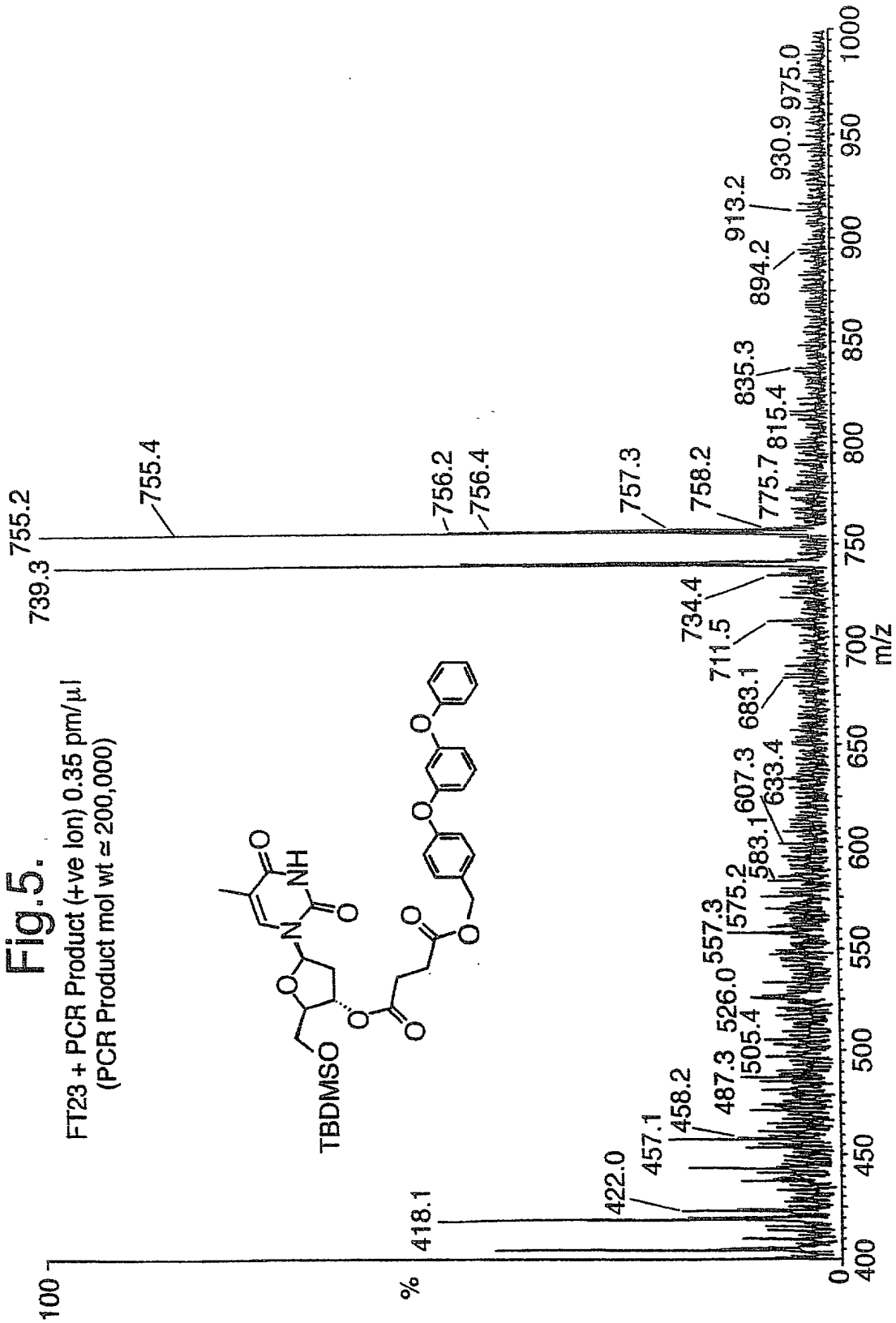
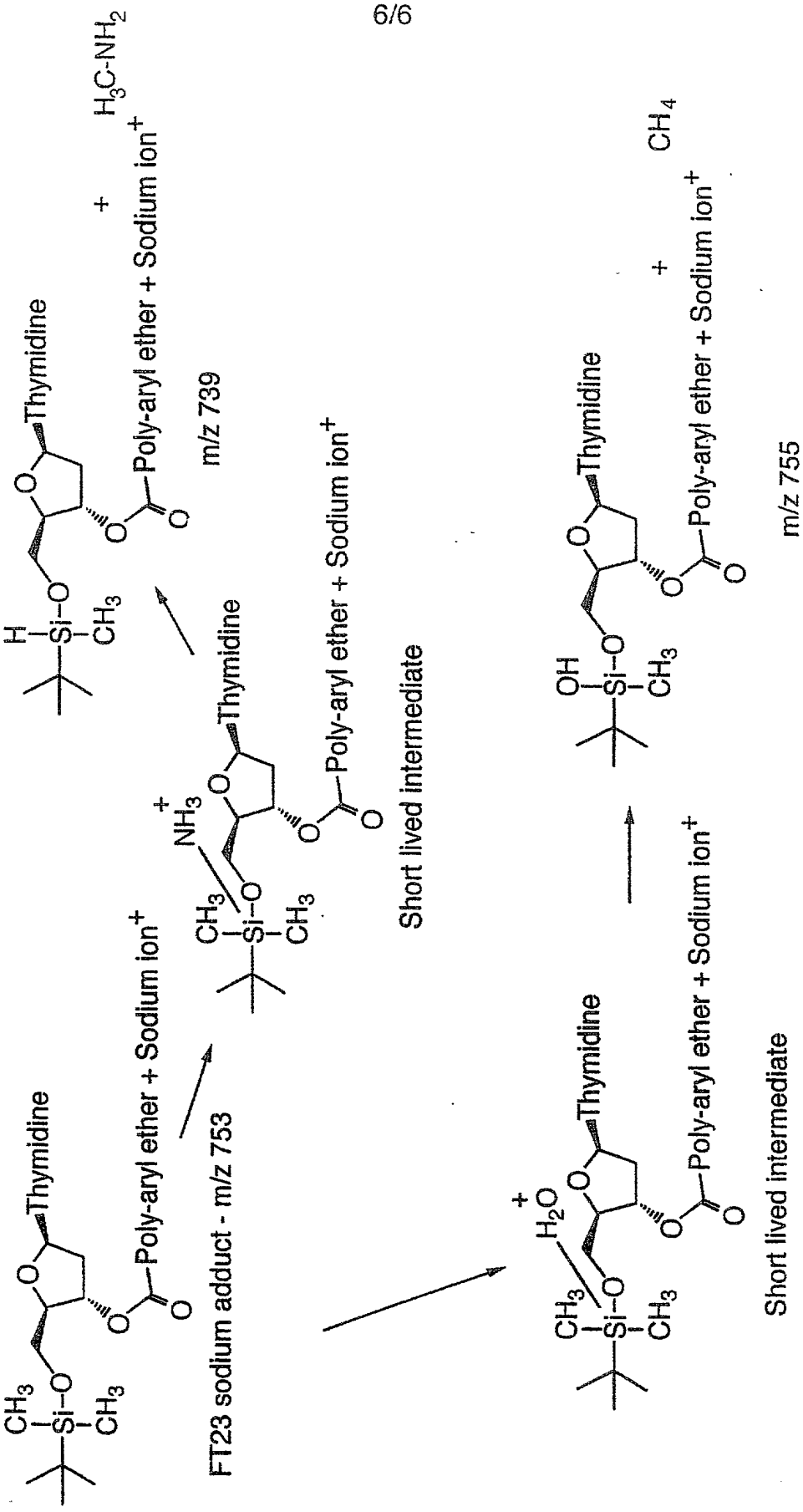


Fig.5.

FT23 + PCR Product (+ve Ion) 0.35 pm/µl
(PCR Product mol wt ≈ 200,000)

Fig.6.

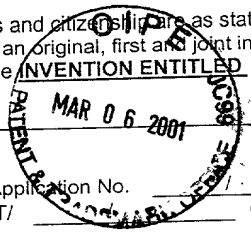

Thymidine
Poly-aryl ether + Sodium ion+
FT23 sodium adduct - m/z 753
Short lived intermediate
m/z 739
H3C-NH2
Short lived intermediate
m/z 755
CH4

FOR UTILITY/DESIGN
CIP/PCT NATIONAL/PLANT
ORIGINAL/SUBSTITUTE/SUPPLEMENTAL
DECLARATIONS

RULE 63 (37 C.F.R. 1.63) DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PW
FORM

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the INVENTION ENTITLED SILICON-CONTAINING LINKERS FOR NUCLEIC ACID MASS MARKERS



the specification of which (CHECK applicable BOX(ES))

X A. ☒ is attached hereto.

BOX(ES) → B. ☒ was filed on January 16, 2001 as U.S. Application No. / on

→ → C. ☐ was filed as PCT International Application No. PCT/ on

and (if applicable to U.S. or PCT application) was amended on

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C F R 1 56 Except as noted below, I hereby claim foreign priority benefits under 35 U.S C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International Application which designated at least one other country than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International Application, filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which priority is claimed, or (2) if no priority claimed, before the filing date of this application:

PRIOR FOREIGN APPLICATION(S) Number	Country	Day/MONTH/Year Filed	Date first Laid-open or Published	Date Patented or Granted	Priority NOT Claimed
PCT/GB99/02247	International	13 July 1999	20 January 2000		
9815164.0	Great Britain	13 July 1998			

If more prior foreign applications, X box *at bottom* and continue on attached page.

Except as noted below, I hereby claim domestic priority benefit under 35 U S.C. 119(e) or 120 and/or 365(c) of the indicated United States applications listed below and PCT international applications listed above or below and, if this is a continuation-in-part (CIP) application, insofar as the subject matter disclosed and claimed in this application is in addition to that disclosed in such prior applications, I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56 which became available between the filing date of each such prior application and the national or PCT international filing date of this application:

PRIOR U.S. PROVISIONAL, NONPROVISIONAL AND/OR PCT APPLICATION(S) Application No. (series code/serial no.)	Day/MONTH/Year Filed	Status pending, abandoned, patented	Priority NOT Claimed

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint Pillsbury Winthrop LLP, Intellectual Property Group, 1100 New York Avenue, N W , Ninth Floor, East Tower, Washington, D C. 20005-3918, telephone number (202) 861-3000 (to whom all communications are to be directed), and the below-named persons (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent, and I hereby authorize them to delete names/numbers below of persons no longer with their firm and to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/ organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct the above Firm and/or a below attorney in writing to the contrary

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Kevin E. Joyce	20508	Lynn E. Eccleston	35861	W. Patrick Bengtsson	32456	Brian J. Beatus	38825
George M. Sirilla	18221	Timothy J. Klima	34852	Jack S. Barufka	37087		
Donald J. Bird	25323	David A. Jakopin	32995	Adam R. Hess	41835		
Dale S. Lazar	28872	Mark G. Paulson	30793	William P. Atkins	38821		
Paul E. White, Jr.	32011	Stephen C. Glazier	31361	Paul L. Sharer	36004		
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27

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2-0

"X" box ☒ FOR ADDITIONAL INVENTORS, and proceed on the attached page to list each additional inventor.

☐ See additional foreign priorities on attached page (incorporated herein by reference).

Atty. Dkt. No. P0276612
(M#)

DECLARATION AND POWER OF ATTORNEY
(continued)
ADDITIONAL INVENTORS:

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3d

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Mailing Address			
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(5) **INVENTOR'S SIGNATURE:** Date:

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Mailing Address			
(include Zip Code)			

(6) **INVENTOR'S SIGNATURE:** Date:

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Residence			
	City	State/Foreign Country	Country of Citizenship
Mailing Address			
(include Zip Code)			

(7) **INVENTOR'S SIGNATURE:** Date:

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Residence			
	City	State/Foreign Country	Country of Citizenship
Mailing Address			
(include Zip Code)			

(8) **INVENTOR'S SIGNATURE:** Date:

	First	Middle Initial	Family Name
Residence			
	City	State/Foreign Country	Country of Citizenship
Mailing Address			
(include Zip Code)			

(9) **INVENTOR'S SIGNATURE:** Date:

	First	Middle Initial	Family Name
Residence			
	City	State/Foreign Country	Country of Citizenship
Mailing Address			
(include Zip Code)			